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The effect of seasonal temperature variation on behaviour and metabolism in the freshwater mussel (*Unio tumidus*)

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abstract

Temperature plays a critical role in determining the biology of ectotherms. Many animals have evolved mechanisms that allow them to compensate biological rates, i.e. adjust biological rates to overcome thermodynamic effects. For low energy-organisms, such as bivalves, the costs of thermal compensation may be greater than the benefits, and thus prohibitive. To examine this, two experiments were designed to explore thermal compensation in *Unio tumidus*. Experiment 1 examined seasonal changes in behaviour in *U. tumidus* throughout a year. Temperature had a clear effect on burrowing rate with no evidence of compensation. Valve closure duration and frequency were also strongly affected by seasonal temperature change, but there was slight evidence of partial compensation. Experiment 2 examined oxygen consumption during burrowing, immediately following valve opening and at rest in summer (24 °C), autumn (14 °C), winter (4 °C), and spring (14 °C) acclimatized *U. tumidus*. Again, there was little evidence of burrowing rate compensation, but some evidence of partial compensation of valve closure duration and frequency. None of the oxygen compensation rates showed any evidence of thermal compensation. Thus, in general, there was only very limited evidence of thermal compensation of behaviour and no evidence of thermal compensation of oxygen compensation rates. Based upon this evidence, we argue that there is no evolutionary pressure for these bivalves to compensate these biological rates. Any pressure may be to maintain or even lower oxygen consumption as their only defence against predation is to close their valves and wait. An increase in oxygen consumption will be detrimental in this regard so the cost of thermal compensation may outweigh the benefits.

Keywords: Thermal Compensationcompensation; Acclimation; Oxygen Consumptionconsumption; Valve Closureclosure; Burrowing

1 Introduction

For cold-blooded, ectothermic animals, environmental temperature plays a determining role in many biological functions. A suite of adjustment mechanisms at different levels of organisation have evolved that allow animals to compensate biological rates, thus retaining function in the face of thermally variable environments (Angilletta, 2009; Tattersall et al., 2012), known as acclimation or acclimatization. An early, but nonetheless practical model of thermal acclimation described five different types of thermal compensation of biological rates that underlie acclimation and acclimatization: supra-optimal; optimal (also known as perfect); partial; no; and inverse compensation (Precht, 1958). Other models have been developed to describe the purpose of acclimation (Angilletta, 2009). The beneficial acclimation hypothesis simply states that "acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that environment" (Leroi and Bennett, 1994, p. 1917). Despite its intuitive sense, data from various experiments have not always supported this hypothesis (Huey and Berrigan, 1996; Wilson, 2007; Wilson and Franklin, 2002), although the argument has subsequently been made that some of the data may be wrongly interpreted due to two confounding factors, namely developmental plasticity, and the detrimental effects of holding animals long-term (see Woods and Harrison, 2002 for more details).

While the beneficial acclimation hypothesis has been tested experimentally in various ways, only a handful of studies, primarily with terrestrial insects, have explicitly examined the costs of acclimation (Hoffmann, 1995; Kristensen et al., 2008; Scott et al., 1997). In field experiments using *Drosophila melanogaster*, clear benefits and costs were demonstrated as a result of both warm and cold acclimation (Kristensen et al., 2008). Direct costs of thermal compensation in aquatic animals, often termed trade-offs in performance, have been examined in various fish species where acclimation to warmer temperatures usually led to enhanced performance at higher temperatures, but with a deterioration at lower temperatures (Pörtner et al., 2006). While the maintenance of performance during temperature changes can be of considerable importance in some fish species, enabling predators to catch prey and prey to escape predators (Catalán et al., 2004; Johnston and Temple, 2002; Rome, 1995), some species adopt a different strategy enhancing the depressive effects of temperature thus reducing metabolic needs to a minimum in times of little (Holopainen et al., 1997), i.e. displaying Precht's inverse compensation.

For bivalves, the ability to burrow and move horizontally from one place to another is of critical importance. For example, *Elliptio complanata* completely bury themselves in autumn and do not emerge until the following spring. This behaviour is thought to reduce the chance of predation, displacement by water currents and wave action, and/or prevent freezing in the shallows where ice may extend to the substrate (Amyot and Downing, 1997). Complete burial must, by necessity, also correlate with valve closure for equally long periods, although this has not yet been quantified. Horizontal locomotion is equally important, for example, during aggregation when spawning, or relocating after disturbance (Amyot and Downing, 1997, 1998). Evidence clearly indicates that both burrowing and locomotion are strongly affected by seasonal changes in temperature. (Amyot and Downing, 1997, 1998; Watters, 2007). Nonetheless, bivalves generally have a very low-energy life-style, exhibiting minimal voluntary mobility and very low athleticism, and remaining largely inactive as they filter water. Although water filtration and the collection of food particles, can account for 90% of metabolic costs (Clemmesen and Jørgensen, 1987), bivalve oxygen consumption rates are typically very low (e.g. Lurman et al., 2014; Tankersley and Dimock, 1993). Based on their low-energy life-style, we expect that bivalves should adopt an energy saving strategy in the face of changing temperatures.

This expectation is further supported by previous studies, for example an early study by Tudorancea and Florescu (1968) that found the oxygen consumption rate of mature *Unio tumidus* to vary directly with season and temperature, while a recent comparison of summer and winter acclimatized *Anodonta anatina* found oxygen consumption, valve opening/closing behaviour (also known as gape), and locomotory speed varied directly with season and temperature, and showed, at best, minimal evidence of thermal compensation (Lurman et al., 2014). In general, oxygen consumption in a range of marine and freshwater bivalves exhibits no thermal compensation at low to intermediate temperatures and only minimal evidence of compensation at intermediate to high temperatures characteristic of their natural thermal range (Alexander and McMahon, 2004; Baker and Hornbach, 2001; Hornbach et al., 1983; Huebner, 1982; Newell et al., 1977; Newell and Pye, 1970; Pernet et al., 2007, 2008; Resgalla et al., 2007; Riascos et al., 2012; Tankersley and Dimock, 1993; Widdows, 1973).

Unio tumidus inhabits a lacustrine habitat where it is subjected to considerable seasonal variation in temperature, from a summer average of 22 °C to a winter average of 6 °C (Lurman et al., 2014). Based on the evidence cited above, it was hypothesised that *U. tumidus* would show minimal, if any evidence of thermal compensation. Two independent experiments were conducted with the aim of examining the effect of seasonal temperature changes on, and the possibility of compensation of behaviour and oxygen consumption in *U. tumidus*. In the first experiment, changes in behavioural parameters, namely burrowing rate, valve closure duration and frequency and the proportion of time spent closed were examined each month throughout one year, with measurements made at a range of temperatures that corresponded to each month.

Based on the results of the first experiment, the energetic costs of burrowing and valve closure were examined in a second experiment in summer, autumn, winter and spring acclimatized mussels. Here, it was also hypothesised that: (1) the resting oxygen consumption rate would be lowest in winter, highest in summer and intermediate in the autumn and spring acclimatized mussels; (2) oxygen consumption during burrowing and immediately after the valves are opened would similarly be lowest in winter, highest in summer and intermediate in the autumn and spring acclimatized mussels; and (3) the Q_{10}s for these rates would range between 2 and 3 and would not show signs of thermal compensation, i.e. acclimatization.

2 Methods

2.1 Mussel ~~Collection~~collection and ~~Holding~~holding

Murtensee water temperature (±0.5 °C) was recorded at 4 hourly intervals between 9 May 2011 and 23 August 2013 using an iButton temperature logger (Embedded Data Systems, Lawrenceburg, USA) at a depth of 1 m ~~(46° 54~~46°54′21.5″~~) "21.5 ~~~~N, ~~7° 30.6 ~" E);~~7°3′0.6″E).

All experiments were conducted in conformity with the “Guiding principles in the care and use of animals” approved by the Council of the American Physiological Society. Given that *U. tumidus* is an endangered species in Switzerland, relevant permits were sought and granted for their use.

Experiment 1 was designed to determine changes in burrowing, valve closure behaviour and locomotion as a result of seasonal temperature changes. *Unio tumidus* and lake sediment for the burrowing experiments were collected by hand in approximately 1 m of water from the same location as above, each month for a year, from May 2011 until April 2012. Mussels were cleaned of epibiota before being transported to the University of Bern (approximately 40 km) in a 2 L sealed box filled with lake water. At the University, they were measured and weighed (Table 2). Mussels were kept in a 60 L aquarium filled with 100% air-saturated, charcoal-filtered, aged tap water at the same temperature as the lake upon collection for a maximum of 3 weeks. Mussels were not fed during this time. To maintain the target temperature within 0.5 °C, the aquarium was kept in a cold-room and heated using a timer controlled 50 W aquarium heater. The water nitrate and nitrite were checked every three days and kept below 0.1 and 10 mg L^{-1}, respectively, with regular water changes.

The total length of time that the individual mussels were observed varied between approximately 1 and 8 days, with longer observation periods required at lower temperatures (see Table 2 for more detail). Artificial lighting followed the natural light-dark cycle, and a low wattage red light was constantly on to allow behavioural observation at night (see below).

Experiment 2 was designed to quantify the energetic cost of burrowing and valve closure using respirometry. Naturally acclimatized mussels were collected from the same location in summer ~~(August – September~~ (August–September 2010), winter ~~(January – March~~ (January–March 2011), autumn ~~(October – November~~ (October–November 2012) and spring ~~(May – June~~ (May–June 2013), at times when the lake temperature approximately matched the desired experimental temperature, i.e. 24±1 °C,

4±1 °C, 14±1 °C and 14±1 ~~°C~~°C, respectively. Mussel collection, transportation and holding were as described above for experiment 1.

2.2 Behavioural ~~Observation~~observation

For experiment 1, random pairs of mussels were allowed to move and burrow voluntarily after being placed in one of three rectangular (210 ~~mm x 100~~mm×100 mm) 1.5 L containers containing autoclaved lake sediment in the aquarium. A webcam connected to a computer that took a time-stamped picture every 30 ~~seconds~~s was used to record behaviour for both experiments. Pictures were compiled into a time-lapse film at a rate of 2 frames per second using Quicktime Player Pro 7.7.3 (Apple Inc., Cupertino, CA). The times at which given activities, namely burrowing, valve closure and opening, as well as locomotory activity, occurred were determined manually.

Three phases involved with burrowing were identified, firstly probing with the foot, then erection into a vertical position, before the actual burrowing typically began. The burrowing duration was defined by the start of the burrowing cycle, once the mussel was in a vertical position and the end, i.e. once the mussel was completely inactive. The burrowing rate index was calculated using formula 1, analogous to Peck et al. (2004):

$$\mathrm{BRI} = \frac{\text{mussel whole weight } (\mathrm{g})^{1/3}}{\text{burrowing duration } (\mathrm{s})} \times 1000 \quad (1)$$

A 20 mm linear black and white scale marked directly on both long edges of the 1.5 L containers allowed for the determination of voluntary locomotory speed. This was calculated from the distance moved along the scale in a given time period.

2.3 Respirometry

For experiment 2, the respirometry chamber, a custom-made chamber of clear plastic with a middle section that could be filled with autoclaved lake sediment, was connected to a 600 L min^{-1} water pump with two one-way valves that allowed the chamber to be flushed with aerated, fully oxygenated water for 2 out of every 30 min using an automatic timer. The total volume of the chamber with sediment, 0.7 L, was determined to be the difference between the weights of the dry and water-filled chamber. The air-saturation of water in the chamber never decreased below 90% during a 30 min measurement interval. Two minutes was sufficiently long to completely recharge the chamber with 100% air-saturated water. The gas-tightness of the chamber was checked regularly by bubbling the water with nitrogen gas and then monitoring oxygen concentration over a 24 h period. A fluorescence oxygen electrode (Model FDO925, WTW, Weilheim, Germany) recorded the water oxygen concentration (in mg L^{-1}) at 30 s intervals. All measurements were automatically temperature compensated.

Before being used for the respirometry experiments, mussels were held for at least 2 days. Mussels of a similar size (Table 2) were used for the determination of oxygen consumption to avoid allometric scaling effects. Individual mussels were selected at random and placed in the chamber, atop the sediment on their side. Mussels were observed and behaviours quantified as per experiment 2. Sediment was autoclaved to ~~minimise~~minimize background oxygen consumption and provided a substrate for burrowing. The mussel was then left in the chamber for 5–7 days and allowed to burrow voluntarily. The drop in the oxygen concentration in the chamber once sealed was equivalent to the mussel's oxygen consumption rate minus background oxygen consumption.

Expression of oxygen consumption per whole mussel (including shell and cavity water), per gram of whole mussel, or per gram wet soft tissue made little difference to the results. To enable comparison with other data, oxygen consumption is expressed here in μg O_2 h^{-1} g soft tissue^{-1}, where the whole animal oxygen consumption rate was divided by the soft tissue wet weight of the mussel. Soft tissue wet weights were calculated from the whole mussel weight, which was derived from a correlation of mussel whole weight and soft tissue wet weight (Eq. (2); ~~r2~~ r^2=0.65, p=0.036, d.f.=84). No significant differences were found between correlations for summer and winter mussels ($F_{2,63}$=2.13, p=0.13), so samples were pooled. Whole mussel weight ranged from 4.13 to 44.34 g.

$$\text{Soft tissue wet weight } (\mathrm{g}) = (0.185 \text{ whole mussel weight}) + 0.73 \quad (2)$$

2.4 Statistics

All analyses were performed using Prism 5.0 (Graphpad). All data were checked for a Gaussian distribution using a ~~Shapiro-Wilk~~Shapiro–Wilk test and proportional data were logit transformed before analysis. For experiment 1, non-parametric one-way ~~Kruskal-Wallis~~Kruskal–Wallis ANOVAs and Dunn's multiple comparison tests were used to test for significant differences in: mussel whole weight, length; BRI; closure frequency; closure duration; and the proportion of time spent closed. Linear models were fit to ~~R-T~~R–T plots of $\log_{10}$ rate (BRI, closure frequency, closure duration) versus temperature (1000/K). For experiment 2, a multivariate analysis was performed on mussel whole weight, whole animal oxygen consumption (mg O_2 h^{-1}), valve closure duration, valve closure frequency and burrowing duration in hours. Significant differences were subsequently checked using one-way ANOVAs and Tukey's multiple comparison tests of the following parameters: mussel weight; mussel length; resting oxygen consumption; oxygen consumption during burrowing; and valve closure duration. Non-parametric one-way ~~Kruskal-Wallis~~Kruskal–Wallis ANOVAs, and Dunn's multiple comparison tests were used to test for significant differences in: BRI; valve closure frequency; proportion of time spent closed; and oxygen consumption after valve closure. All values are presented as the mean±the standard error of the mean.

3 Results

3.1 Lake ~~Temperature~~temperature

Lake temperature at 1 m changed as a result of season (Fig. 1). The maximum temperature measured on 6 Aug 2013 was 28.9 °C and the minimum was 0.97 °C in February 2012 and 2013, with the water having frozen and mussels retreating into the lake sediment. A frequency distribution of temperature measurements revealed a bimodal distribution with peaks at 21.4±0.47 and 5.9±0.19 °C ~~(Supplementary~~(Supplementary Fig. 1~~ Figure 1),~~), representing the summer and winter ~~averages~~averages, respectively. Seasonal rates of temperature change were approximately 0.2 °C per day in spring and autumn. Considerable variation was also seen on shorter scales particularly in summer and winter, with rates of change as high as 1–2 °C d~~-1~~$^{-1}$ for several days running, although diel variation was low.

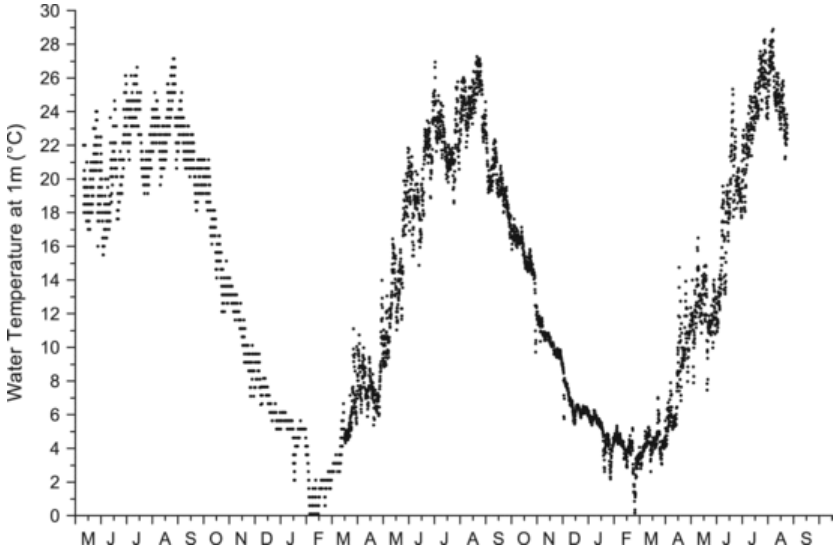


Fig. 1 Seasonal changes in water temperature in Lake Murten (Murtensee) at 1 m depth ~~(46° 54′ 24″ N, 7° 2′ 59″ E)~~ (46°54′24″N, 7°2′59″E) from 9 May 2011 until 23 August 2013.

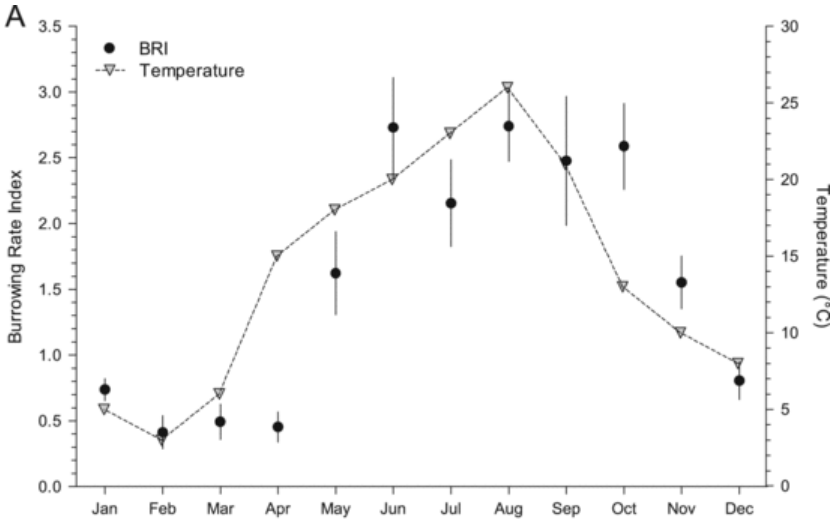
3.2 Experiment 1: Seasonal ~~Changes~~changes in ~~Behaviour~~behaviour

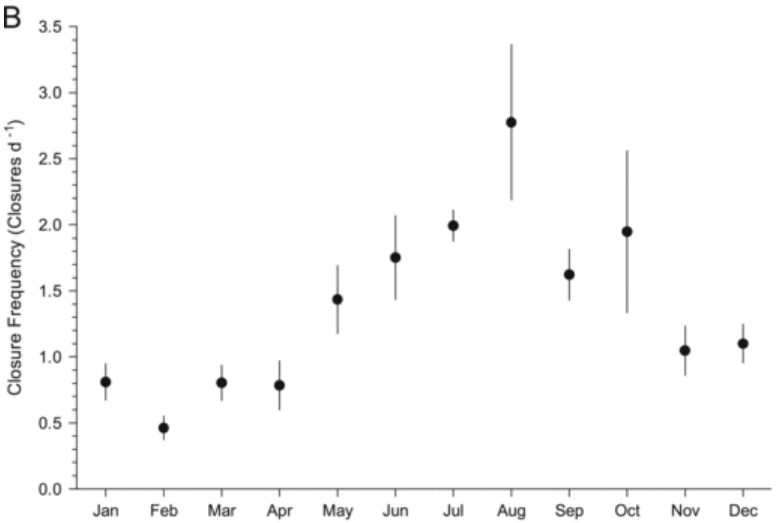
Mussel whole weight (Kruskal–Wallis: $H_{~~1~~11}$=23.7, p=0.014) and length (Kruskal–Wallis: $H_{~~1~~11}$=32.7, p<0.01) differed significantly between months. This was due to a significant difference between April and July *U. tumidus* (Table 1). There was a statistically significant effect of month on BRI (Kruskal–Wallis: $H_{~~1~~11}$=69.2, p<0.01), valve closure frequency (Kruskal–Wallis: $H_{~~1~~11}$=51.6, p<0.01), valve closure duration (Kruskal–Wallis: $H_{~~1~~11}$=30.2, p<0.01), and the proportion of time *U. tumidus* spent closed (Kruskal–Wallis: $H_{~~1~~11}$=50.7, p<0.01). In general, summer acclimatized *U. tumidus* burrowed faster and closed more often, but for shorter durations (Fig. 2A–C), while the proportion of time spent closed was only significantly different in February and August, the two months with the most extreme temperatures (Fig. 3). Pairwise comparisons of individual months (see Supplementary Table 1 for details) revealed statistically significant differences between summer (primarily June, July and August) and winter months (January, February and March). Noteworthy was the significant difference in BRI between April and October, where the temperature was similar (15 °C in April and 13 °C in October), yet the BRI was more than 5-fold lower in April. This was also reflected, albeit to a lesser degree, in the valve closure frequency and duration, which were both more than 2-fold lower in April.

Table 1 The whole mussel weights (including the valves) and lengths (mean±SEM) and the number of mussels (*N*) used for each of the derived parameter. Significant differences were seen for weight (Kruskal–Wallis: $H_{~~1~~11}$=23.7, p=0.014) and length (Kruskal–Wallis: $H_{~~1~~11}$=32.7, p<0.01).

Month	Mean ~~Weight~~weight (g)	Mean ~~Length~~length (mm)	Observation ~~Period~~period (d)	*N* (total)	Burrowing	Valve ~~Closure~~closure
Jan	22.0±1.2	57.4±1.2	2.02±0.29	17	16	6
Feb	21.3±2.0	56.4±2.0	8.32±0.91	13	4	9
Mar	18.0±2.6	51.6±2.8	2.21±0.42	13	8	5
Apr	14.4±2.3	45.5±3.3	4.24±0.55	13	10	10
May	20.0±3.5	52.9±4.0	2.04±0.24	10	10	8
Jun	20.9±2.4	53.9±2.1	0.96±0.04	27	22	10
Jul	27.6±2.7	59.8±1.6	1.60±0.20	17	14	16

Aug	23.0±2.3	57.9±2.1	2.52±0.61	14	11	14
Sep	21.2±2.5	56.6±2.3	2.76±0.55	13	13	6
Oct	24.3±2.2	60.4±1.9	1.08±0.34	16	16	6
Nov	28.0±3.5	59.1±2.1	2.78±0.86	16	11	5
Dec	16.3±2.1	47.6±2.6	1.15±0.10	16	12	7





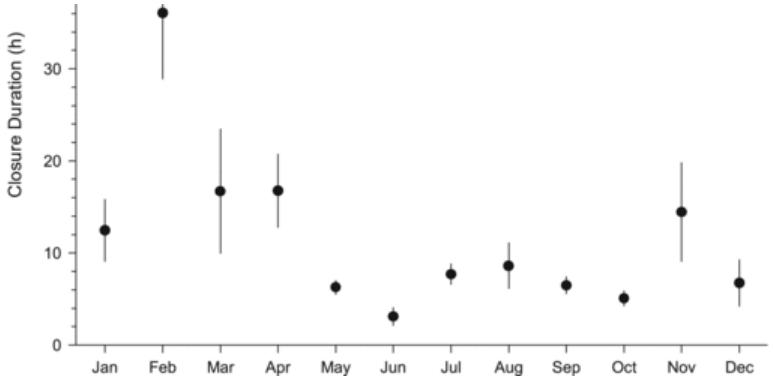


Fig. 2 Seasonal changes in burrowing rate index (A), valve closure frequency (B), and valve closure duration (C). The temperature used each month is also given on panel A. Month had a significant effect on the burrowing rate index (Kruskal–Wallis: $H_{11,11}$=69.2, p<0.01), the valve closure frequency (Kruskal–Wallis: $H_{11,11}$=51.6, p<0.01), and valve closure duration (Kruskal–Wallis: $H_{11,11}$=30.1, p<0.01). For a complete summary of the pairwise comparisons of months, see Supplementary Table 2. Correlation coefficients (r^2 values) for the correlation with temperature were 0.64 ($F_{(1,10)1,10}$=17.8, p<0.01) for burrowing rate index, 0.73 ($F_{(1,10)1,10}$=27.1, p<0.01) for valve closure frequency, and 0.39 ($F_{(1,10)1,10}$=6.27, p=0.03) for valve closure duration.

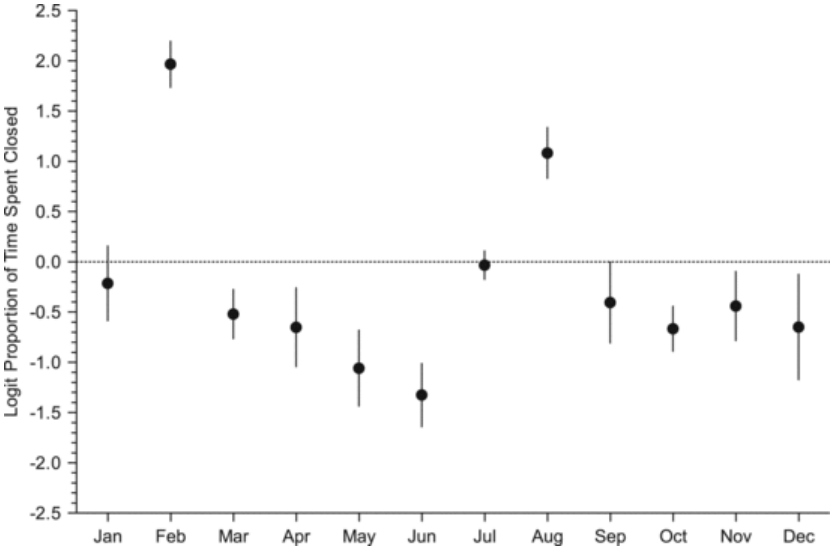


Fig. 3 Seasonal changes in the proportion of time *Unio tumidus* spent closed. The temperature used each month is also given in Fig. 2A. Month had a significant effect on the proportion of time spent closed (Kruskal–Wallis: $H_{11,11}$=50.7, p<0.01).

Correlation coefficients (r^2 values) for the correlation of burrowing rate index, valve closure frequency and valve closure duration with temperature were 0.64 ($F_{(1,10)1,10}$=17.8, p<0.01) for burrowing rate index, 0.73 ($F_{(1,10)1,10}$=27.1, p<0.01) for valve closure frequency, and 0.39 ($F_{(1,10)1,10}$=6.27, p=0.03) for valve closure duration. Linear models were fit to each of the different $\log_{10}$ transformed data sets on R–T plots. The derived descriptors of each of these fits are given in Table 3. Briefly, a slope of 0 is equivalent to a Q_{10} of 1, or Precht's perfect compensation, slopes between 0 and ±2.56 are equivalent to Q_{10}s of 1–2, indicative of Precht's partial compensation, and slopes between ±2.56 and ±4.05 are equivalent to Q_{10}s of 2 and 3, respectively, indicative of no compensation. For the BRI, the slope approximated an idealized R–T plot slope indicative of non-compensation, while the valve closure frequency and duration slope values were closer to an idealized R–T plot slope indicative of partial compensation.

Table 2 Morphometric data from mussels used for the determination of oxygen consumption in experiment 2. Whole mussel weight includes the valves. Values are means±SEM.

Parameter	Summer	Autumn	Winter	Spring
Whole mussel weight (g)	19.3±2.06	17.4±1.50	17.3±2.22	20.4±0.51
Length (mm)	53.1±2.03	52.3±1.58	51.1±2.13	56.1±0.67

~~N~~N	8	9	8	8

Table 3 Descriptive parameters (mean±SEM) derived from the linear model fits to ~~R~~R–T transformed data of rate (log_{10} of rate) versus temperature (1000/K) from experiment 1.

Rate	Slope	Intercept	*p* ~~P value~~Value	~~r~~r^2 value
BRI	–2.63±0.78	9.23±2.7	0.007	0.53
Valve closure frequency (closures d~~-1~~**)**	–1.92±0.44	6.77±1.5	0.001	0.66
Valve closure duration (h)	1.89±0.63	–5.73±2.2	0.013	0.48

3.3 Experiment 2: Energetic ~~Cost~~cost of ~~Burrowing~~burrowing and ~~Valve Closure~~valve closure

There were no significant differences in the whole mussel weight (Kruskal–Wallis: H_{-3}=3.50, p=0.32) of (or) length (Kruskal–Wallis: H_{-3}=5.99, p=0.11) between summer, autumn, winter and spring groups (Table 2), and a multivariate analysis found whole weight did not significantly co-vary with whole animal oxygen consumption, valve closure duration, valve closure frequency nor burrowing duration in hours (Pillai's Trace: $F_{(4,17)-17}$=2.59, p=0.074). The BRI was significantly higher in the summer acclimatized *U. tumidus* compared to both the winter and spring *U. tumidus* by a factor of 3.3 and 3.0, respectively, (Kruskal–Wallis: H_{-11}=20.9, p<0.01 Fig. 4). The Q_{10}s for burrowing are given in Table 4. Valve closure frequency differed significantly between seasons (Kruskal–Wallis: H_{-11}=14.9, p<0.01 Fig. 5A). Valve closure frequency was 3.5, 3.5 and 2.4 times lower in the summer *U. tumidus* compared to the autumn, winter and spring *U. tumidus*, respectively. No significant differences between closure durations were seen as a result of season (ANOVA: $F_{(3,29)-3.29}$=1.90, p=0.15 Fig. 5B), where valve closure duration was 2.4 and 1.2 times lower, respectively, and 1.2 times higher in the spring *U. tumidus*. The proportion of time *U. tumidus* spent closed was not significantly different between groups (Kruskal–Wallis: H_{-11}=4.90, p<0.18).

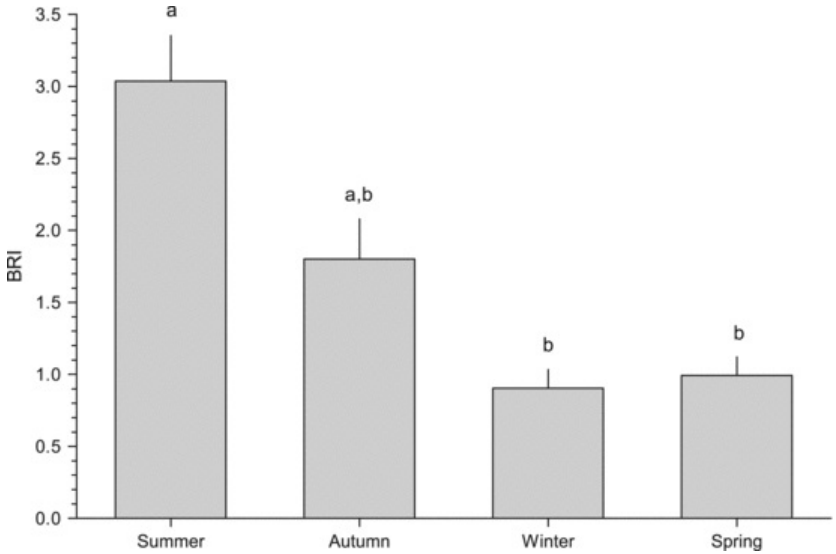


Fig. 4 The burrowing rate index (BRI) in summer (24 °C), autumn (14 °C), winter (4 °C) and spring (14 °C) acclimatized *Unio tumidus*. Significant differences between seasons (where p<0.05) are indicated by different letters.

Table 4 The Q_{10} values for burrowing, valve closure and oxygen consumption from experiment 2.

~~Q~~Q_{10} values	BRI	Closure frequency	Closure duration	MO_2-burrowing	MO_2-valve closure	MO_2-rest
~~Summer-Autumn~~Summer–Autumn	1.69	3.48	4.51	2.13	1.60	2.14
~~Summer-Winter~~Summer–Winter	1.83	1.86	1.64	2.37	1.96	2.43
~~Summer-Spring~~Summer–Spring	3.07	2.44	1.94	1.96	2.09	1.45
~~Autumn-Winter~~Autumn–Winter	1.99	1.00	0.60	2.63	2.41	2.74

~~Spring–Winter~~Spring–Winter	1.10	1.42	1.39	2.86	1.84	4.06

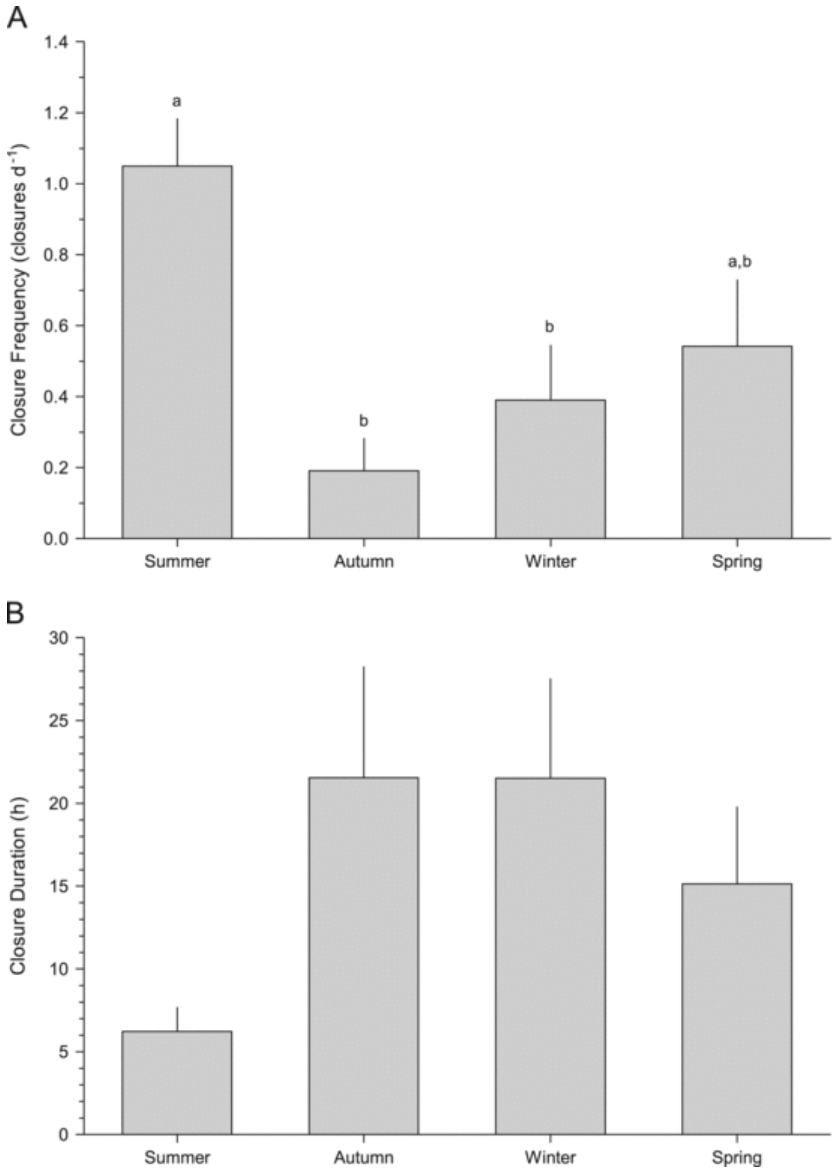


Fig. 5 The valve closure frequency (A) and valve closure duration (B) in summer (24 °C), autumn (14 °C), winter (4 °C) and spring (14 °C) acclimatized *Unio tumidus*. Significant differences between seasons (where $p<0.05$) are indicated by different letters.

Oxygen consumption was significantly affected by seasonal acclimatization (ANOVA: $F_{(3,27)}$=11.4, $p<0.01$ for MO_2 during burrowing, Kruskal–Wallis: $H_{[3]}$=17.9, $p<0.01$ for MO_2 after valve closure, and ANOVA: $F_{(3,29)}$=15.2, $p<0.01$ for resting MO_2 Fig. 6A–C). During burrowing, oxygen consumption in summer *U. tumidus* was significantly higher than autumn, winter and spring *U. tumidus* by a factor of 2.2, 5.7 and 2.0, respectively. The oxygen consumption rate immediately after valve closure was also highest in summer *U. tumidus* by a factor of 1.6, 3.9 and 2.1 compared to autumn, winter and spring *U. tumidus*, respectively, however this was only statistically significant for the summer/winter *U. tumidus* comparison.

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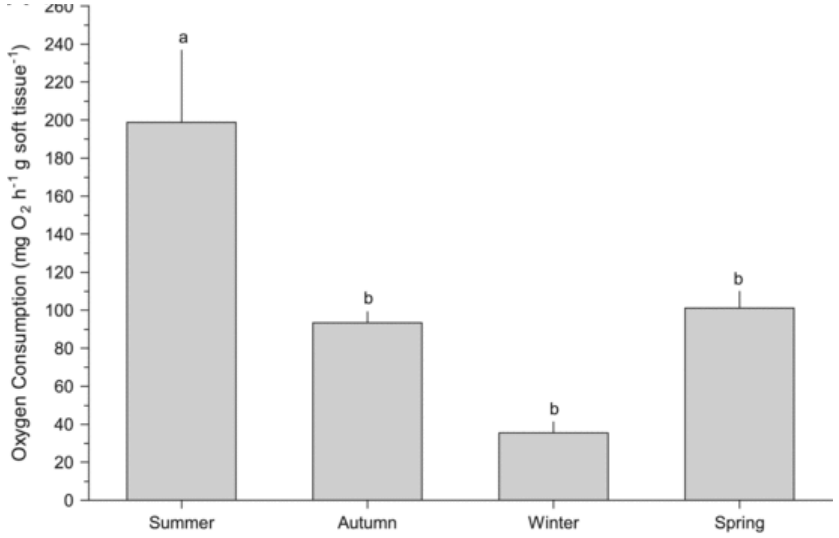
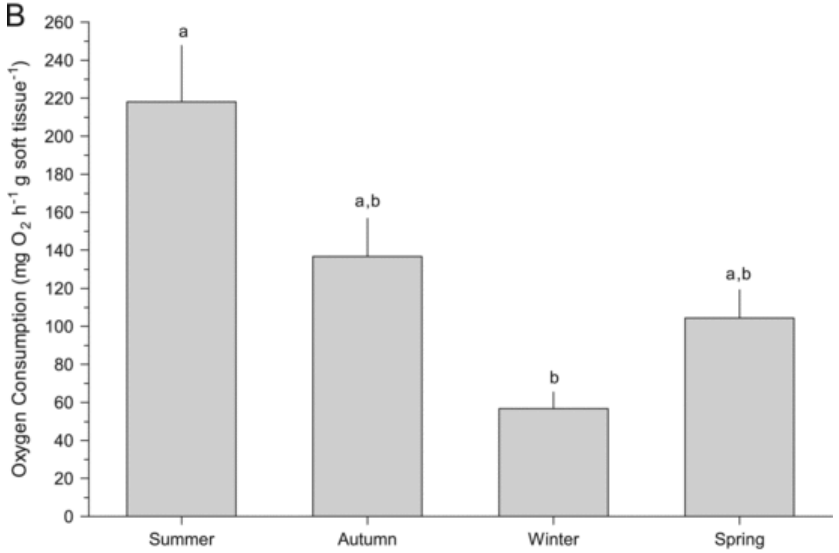


B



C

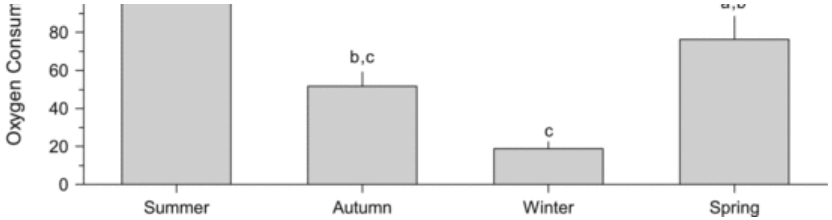


Fig. 6 The oxygen consumption rate (MO_2) during burrowing (A), immediately after valve closure (B), and at resting (C) in summer (24 °C), autumn (14 °C), winter (4 °C) and spring (14 °C) acclimatized *Unio tumidus*. Significant differences between seasons (where $p<0.05$) are indicated by different letters.

Oxygen consumption at rest was again highest in summer *U. tumidus* by a factor of 2.2, 5.9 and 1.4 compared to autumn, winter and spring *U. tumidus*, respectively. These differences were statistically significant for summer *U. tumidus* compared to autumn and winter *U. tumidus* and for spring compared to winter *U. tumidus*. Generally speaking, significant differences were observed in all three rates of oxygen consumption, primarily between summer and winter acclimatized *U. tumidus*, however, no statistically significant differences were seen between autumn and spring acclimatized *U. tumidus*.

Significant differences between oxygen consumption were also seen within acclimatization groups as a result of burrowing and valve closure in the summer (ANOVA: $F_{(2,21)}$=3.96 p=0.03), autumn (Kruskal–Wallis: H_{2}=13.9, $p<0.01$) and winter (Kruskal–Wallis: H_{3}=9.66, $p<0.01$) groups. In all three groups the difference was most pronounced between the oxygen consumption rate after valve closure and at rest. Significant differences were not seen in the spring *U. tumidus* as a result of burrowing and valve closure where oxygen consumption during burrowing and immediately after valve closure were 1.3 and 1.4 times higher, respectively, than resting oxygen consumption (Kruskal–Wallis: H_{3}=3.44, p=0.18). This appears to be due largely to an elevated resting oxygen consumption rate.

4 Discussion

4.1 Thermal ~~Compensation~~compensation

As expected, and similar to *A. anatina* from the same habitat and the same lake (Lurman et al., 2014), there was a strong effect of temperature on the behaviour of *U. tumidus* throughout the year with minimal thermal compensation. Winter led to slower burrowing, less frequent opening and closing and a longer duration of closing. For all three behavioural parameters, there was a significant correlation with temperature, however the strength of the correlation varied. Temperature explained 64% of the variation in the BRI and 73% of the variation in valve closure frequency, but only 35% of the variation in closure duration. Examination of the R–T slopes for these parameters (Table 3) shows that there was no evidence of thermal compensation for BRI, however there was some evidence for partial compensation of the valve closure frequency and duration. Also worth noting was the pronounced difference in BRI in spring (April) and autumn (October) *U. tumidus*, despite being measured at approximately the same temperature, i.e. 15 and 13 °C, respectively. This significant difference provoked an examination of the oxygen consumption during burrowing in spring and autumn *U. tumidus* in experiment 2.

A comparison of the R–T plot slope values for the different behaviours from experiment 1 (Figs. 4 and 5), with the Q_{10} values from experiment 2 (Table 4), indicate that in general there was only marginal evidence of thermal compensation of behaviour, while oxygen consumption did not show any signs of thermal compensation. There are two exceptions to this. In experiment 1, summer, autumn and winter *U. tumidus* did not compensate their burrowing rate, however, spring *U. tumidus* compensated with an unusually slower burrowing rate, similar to what was seen in the experiment 2. Second, with respect to valve closure duration, in experiment 2 it was summer, winter and spring *U. tumidus* that did not show signs of thermal compensation, but autumn *U. tumidus* had a longer closure duration and a lower frequency, indeed even lower than winter *U. tumidus*.

Factors responsible for the lower than thermodynamically predicted burrowing rate may include: inter-annual effects, i.e. the fact that this study was carried out over 2 years may have potentially impacted upon behaviour and/or oxygen consumption rates, however, the temperature logs indicate that the season changes were very similar; structural changes at a molecular and cellular level; a simple behavioural response; or an energetic limitation due to depleted substrate stores after winter, although the oxygen consumption data do not lend support this final proposition. Similarly, the enhanced valve closure activity observed in the autumn mussels observed in experiment 2 may be an energy saving mechanism, as has previously been seen in the Antarctic mud clam *Laternula elliptica* in times of food scarcity (Peck, 1998). In addition to this, late autumn may be the time of a pause in the reproductive cycle, as seen in *Unio terminalis* (Çek and Şereflişan, 2006) and ~~Elliptic~~*E. complanata* (Amyot and Downing, 1997), resulting in decreased valve activity.

Reproduction, most notably gametogenesis, is known to impact significantly upon oxygen consumption rates and energy budgets in marine bivalves (Jansen et al., 2007; Sukhotin, 1992; Urrutia et al., 1999; Widdows, 1978). In a previous study, the oxygen consumption in mature *U. tumidus* changed in direct accordance with temperature (Tudorancea and Florescu, 1968), similar to what we report here, and elsewhere for *A. anatina* from the same area (Lurman et al., 2014). Valve opening and closing behaviour is also known to be affected by reproduction, with one North American freshwater bivalve, *Pyganodon cataracta*, displaying increased “valve activity” in winter due to glochidial brooding (Tankersley and Dimock, 1993). Due to the endangered nature of *U. tumidus*, we did not make efforts to determine the gonado-somatic index, nor determine whether females were gravid. As such, we cannot rule out the possibility that the reproductive state may have influenced behaviour and/or oxygen consumption, although

we see no evidence indicating that it did.

While evidence of compensation of oxygen consumption has previously been reported in intertidal bivalves, these data represent a different situation to the one observed here for *U. tumidus*. For example, standard and active oxygen consumption in *Mytilus edulis* show partial thermal compensation (Q_{10}s of 1 to 2.1) immediately following an acute temperature change, yet 21 days of acclimation at the same temperatures resulted in a very similar degree of metabolic compensation (Q_{10}s of 1.3 to 1.7) (Widdows, 1973). Thus, the compensation of oxygen consumption occurred immediately upon temperature change and not as a result of acclimation. Data from other marine and freshwater bivalves such as *Perna perna* (Resgalla et al., 2007), *Dreissena polymorpha* (Alexander and McMahon, 2004), *Amblema* sp. (Baker and Hornbach, 2001), clearly indicate that oxygen consumption can be partially compensated following acclimation/acclimatization to intermediate temperatures, but not to extreme temperatures. For example, the freshwater zebra mussel *D. polymorpha* shows partial compensation between 15 and 25 °C (Q_{10}≈1.6), but not between 15 and 5 °C (Q_{10}≈2.7) (Alexander and McMahon, 2004), while Q_{10} values for *Amblema plicata* were 1.5 and 1.6 at 11–20 °C and 11–25 °C, respectively, but outside of this range, e.g. 5–11 °C or 20–25 °C, Q_{10}s were 2 or greater (Baker and Hornbach, 2001). This correlates with our behavioural observation of significant and substantial increases in the proportion of time *U. tumidus* spent closed which coincided with the most extreme temperatures, i.e. in February (2–3 °C) and Summer (26–27°). Thus, increased valve closure in freshwater bivalves is a protection mechanism during times of stress, as previously seen when exposed to acutely elevated temperatures (Rodland et al., 2009), or adverse conditions such as low water oxygen concentration (Chen et al., 2001), or low pH (Pynnönen and Huebner, 1995).

At the same time however, there is evidence from ~~*Mytilus*~~*M. edulis* that clearance rates are not thermally compensated (Kittner and Riisgård, 2005), indicating that different physiological functions may be affected differently by temperature changes. Thus, although the body of evidence presented here for *U. tumidus* shows little evidence of thermal compensation, compensation of other functions or compensation at a cellular or molecular level cannot be ruled out. Indeed, there is a large body of work that has explored cellular and molecular thermal compensation mechanisms in aquatic species (see Angilletta, 2009; Pörtner, 2010; Tattersall et al., 2012 for reviews), most notably fishes (Godiksen and Jessen, 2002; Guderley, 2004b; Hochachka and Somero, 1984; Johnston et al., 1998; Keen and Farrell, 1994; McArdle and Johnston, 1982; Rome, 1990; Sidell, 1983). Combined, these factors help maintain swimming performance in the face of thermal variation, and thus maintain the ability to catch prey or out-swim predators. Although these phenomena have not been as well explored in aquatic invertebrates, changes are known to occur in mitochondrial structure (Lurman et al., 2010a,b) and the membrane lipid composition (Pernet et al., 2007) as a result of acclimatization. While at the whole animal level, this can result in changes in (scallop) swimming mechanics (Bailey and Johnston, 2005) and recovery duration from swimming (Guderley, 2004a), or the acute thermal tolerance limits in freshwater mussels (Galbraith et al., 2012).

4.2 Ecological ~~Significance~~significance of ~~Not Compensating~~not compensating

The question remains, however, why is it that these mussels show only very limited signs of thermal compensation given the significant thermal variability of Lake Murten. Indeed, current theories would predict that such variability provides ideal conditions for such plasticity to evolve (Angilletta, 2009; Gabriel and Lynch, 1992). The short answer may be that there is simply no selective pressure to thermally compensate, or the flip-side, there may be selective pressure to not acclimate. With respect to being prey, there is no evidence to suggest that *U. tumidus* is preyed upon in Lake Murten, thus there is no need for them to "out-run" a predator. Indeed, this could hardly be expected of an animal whose top speed is less than 1 cm min^{-1}. The most effective strategy to avoid predation is to simply close the valves and wait. Any increase in oxygen consumption as a result of thermal compensation would be of obvious disadvantage. Thus, if there is any selective pressure, it would be to keep oxygen consumption as low as possible. With respect to capturing prey, *U. tumidus* is a filter feeder, feeding upon algae collected with its' gills. Increasing food capture rates does not require an enormous expenditure of energy to chase prey items. Indeed the cost of pumping for filter feeding bivalves was estimated to be 1.6% of the total energetic costs (Jørgensen et al., 1986). However, there is little reason to increase filtration rates in winter given that algal concentrations are generally low at this time. Therefore, being neither predator nor prey, there is little for *U. tumidus* to gain by increasing locomotory ability, because the associated increase in oxygen consumption rate would impinge upon their ability to close their valves when threatened.

Furthermore, the ability of *U. tumidus* to keep their valves closed for very long periods is a prerequisite for their ability to remain completely buried for equally long periods. In late autumn *E. complanata* burrow completely and it is thought that they remain buried until spring (Amyot and Downing, 1997). This may be to avoid freezing in the shallows where ice can extend to the lake bottom. In the shallows of Lake Murten, ice cover reaches the lake bottom on occasion, and we have regularly recorded slightly warmer temperatures (+2–3 °C) in the sediment than the water column in winter. Thus, the lake bottom may act as a thermal retreat for *U. tumidus* in winter. This ~~also~~ is also supported by the fact that it is extremely rare to find *U. tumidus* protruding from the lake sediment in winter, with most buried completely. Similar to our previous study of *A. anodonta* (Lurman et al., 2014), we conclude that maintaining an oxygen consumption rate as low as possible would be of obvious advantage to *U. tumidus*, as it would minimize the frequency and/or duration with which they would need to open their valves to respire. Thus, in many respects the costs of thermal compensation, i.e. acclimation/acclimatization, must outweigh the benefits in this low-energy species.

~~Acknowledgements:~~Acknowledgements

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2014.04.005.

References

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Alexander J.E., ~~Jr~~Jr. and McMahon R.F., Respiratory response to temperature and hypoxia in the zebra mussel *Dreissena polymorpha*, *Comp. Biochem. Physiol. Part A: Mol. ~~& Integ. Physiol~~Integr. Physiol.* **137**, 2004, 425–434.

Amyot J.-P. and Downing J., Seasonal variation in vertical and horizontal movement of the freshwater bivalve *Elliptio complanata* (Mollusca: Unionidae), *~~Freshwat. Biol~~Freshwater Biol.* **37**, 1997, 345–354.

Amyot J.P. and Downing J.A., Locomotion in *Elliptio complanata* (Mollusca: Unionidae): a reproductive function?, *~~Freshwat. Biol~~Freshwater Biol.* **39**, 1998, 351.

Angilletta M.J., ~~Jr~~Jr., (I'm not sure whether the formatting is entirely correct here. The authors surname is Angiletta Jr. The same applies for Resgalla.)Thermal Adaptation: A Theoretical and Emperical Synthesis, 2009, Oxford University Press; Oxford.

Bailey D.M. and Johnston I.A., Scallop swimming kinematics and muscle performance: ~~Modelling~~modelling the effects of ~~"within-animal"~~"within-animal" variation in temperature sensitivity, *Mar. ~~Freshwat.~~Freshwater Behav. ~~Physiol~~Physiol.* **38**, 2005, 1–19.

Baker S.M. and Hornbach D.J., Seasonal metabolism and biochemical composition of two unionid mussels, *Actinonaias ligamentina* and *Amblema plicata*, *J. Molluscan ~~Stud~~Stud.* **67**, 2001, 407–416.

Catalán I.A., Johnston I.A. and Olivar M.P., Seasonal differences in muscle fibre recruitment of pilchard larvae in the north-western Mediterranean, *J. Fish Biol.* **64**, 2004, 1605–1616.

Çek Ş. and Şereflişan H., Certain reproductive characteristics of the freshwater mussel *Unio terminalis delicates* (Lea, 1863) in Gölbaşı Lake, Turkey, *Aquacult. Res.* **37**, 2006, 1305–1315.

Chen L.-Y., Heath A.G. and Neves R.J., Comparison of oxygen consumption in freshwater mussels (Unionidae) from different habitats during declining dissolved oxygen concentration, *Hydrobiologia* **450**, 2001, 209–214.

Clemmesen B. and Jørgensen C.B., Energetic costs and efficiencies of ciliary filter feeding, *Mar. Biol.* **94**, 1987, 445–449.

Gabriel W. and Lynch M., The selective advantage of reaction norms for environmental tolerance, *J. Evol. ~~Biol~~Biol.* **5**, 1992, 41–59.

Galbraith H.S., Blakeslee C.J. and Lellis W.A., Recent thermal history influences thermal tolerance in freshwater mussel species (Bivalvia: Unionoida), *~~Freshwat. Sci~~Freshwater Sci.* **31**, 2012, 83–92.

Godiksen H. and Jessen F., Temperature and Ca^{2+}-dependence of the sarcoplasmic reticulum Ca^{2+}-ATPase in haddock, salmon, rainbow trout and zebra cichlid, *Comp. Biochem. ~~Physiol.~~Physiol. Part ~~B~~B: Biochem. Mol. Biol.* **133**, 2002, 35–44.

Guderley H., Locomotor performance and muscle metabolic capacities: Impact of temperature and energetic status, *Comp. Biochem. ~~Physiol.~~Physiol. Part ~~B~~B: Biochem. Mol. Biol.* **139**, 2004a, 371–382.

Guderley H., Metabolic responses to low temperature in fish muscle, *Biol. Rev.* **79**, 2004b, 409–427.

Hochachka P.W. and Somero G.N., Biochemical Adaptation, 1984, Oxford University Press; Oxford.

Hoffmann A.A., Acclimation: increasing survival at a cost, *Trends Ecol. Evol.* **10**, 1995, 1–2.

Holopainen I.J., Tonn W.M. and Paszkowski C.A., Tales of two fish: the dicotomous biology of crucian carp (*Carassius carassius* (L.)) in northern Europe, *Ann. Zool. Fenn.* **34**, 1997, 1–22.

Hornbach D.J., Wissing T.E. and Burky A.J., Seasonal variation in the metabolic rates and Q_{10}-values of the fingernail clam, *Sphaerium striatinum* lamarck, *~~Sphaerium striatinum~~Comp. Biochem. Physiol. Part A: Physiol.* **76**, 1983, 783–790.

Huebner J.D., Seasonal variation in two species of unionid clams from Manitoba, Canada: respiration, *Can. J. Zool.* **60**, 1982, 560–564.

Huey R.B. and Berrigan D., Testing evolutionary hypotheses of acclimation, In: Johnston I.A. and Bennett A.F., (Eds.), *Phenotypic and ~~evolutionary adaptation~~Evolutionary Adaptation to ~~temperature~~Temperature*, 1996, University of Cambridge Press; Cambridge, 205–237.

Jansen J.M., Pronker A.E., Kube S., Sokolowski A., Sola J.C., Marquiegui M.A., Schiedek D., Bonga S.W., Wolowicz M. and Hummel H., Geographic and seasonal patterns and limits on the adaptive response to temperature of european *Mytilus* spp. and *Macoma balthica* populations, *Oecologia* **154**, 2007, 23–34.

Johnston I.A., Calvo J., Guderley H., Fernandez D. and Palmer L., Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes, *J. Exp. Biol.* **201**, 1998, 1–12.

Johnston I.A. and Temple G.K., Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour, *J. Exp. Biol.* **205**, 2002, 2305–2322.

Jørgensen C.B., Famme P., Kristensen H.S., Larsen P.S., Møhlenberg F. and Riisgård H.U., The bivalve pump, *Mar. Ecol. Prog. Ser.* **34**, 1986, 69–77.

elsevier_TB_1516

Keen J.E. and Farrell A.P., Maximum prolonged swimming speed and maximum cardiac performance of rainbow trout, *Oncorhynchus mykiss*, acclimated to two different water temperatures, *Comp. Biochem. ~~Physiol.,~~Physiol. Part ~~A, Physiol~~A: Physiol.* **108**, 1994, 287–295.

Kittner C. and Riisgård H.U., Effect of temperature on filtration rate in the mussel *Mytilus edulis*: no evidence for temperature compensation, *Mar. Ecol. Prog. Ser.* **305**, 2005, 147–152.

Kristensen T.N., Hoffmann A.A., Overgaard J., Sørensen J.G., Hallas R. and Loeschcke V., Costs and benefits of cold acclimation in field-released *Drosophila*, *Proc. Nat. Acad. Sci. ~~U.S.A~~U.S.A.* **105**, 2008, 216–221.

Leroi A.M. and Bennett A.F., Temperature acclimation and competitive fitness: ~~An~~an experimental test of the beneficial acclimation assumption, *Proc. Nat. Acad. Sci. ~~U.S.A~~U.S.A.* **91**, 1994, 1917–1921.

Lurman G., Blaser T., Lamare M., Peck L. and Morley S., Mitochondrial plasticity in brachiopod (*Liothyrella* spp.) smooth adductor muscle as a result of season and latitude, *Mar. Biol.* **157**, 2010a, 907–913.

Lurman G., Blaser T., Lamare M., Tan K.-S., Poertner H., Peck L. and Morley S., Ultrastructure of pedal muscle as a function of temperature in nacellid limpets, *Mar. Biol.* **157**, 2010b, 1705–1712.

Lurman G.J., Walter J. and Hoppeler H., Seasonal changes in the behaviour and respiration physiology of the freshwater duck mussel *Anodonta anatina*, *J. Exp. Biol.* **217**, 2014, 235–243.

McArdle H.J. and Johnston I.A., Temperature adaptation and the kinetics of the Ca^{2+}-independent and Ca^{2+}-dependent ATPases of fish sarcoplasmic reticulum, *J. Therm. Biol.* **7**, 1982, 63–67.

Newell R.C., Johson L.G. and Kofoed L.H., Adjustment of the components of energy balance in response to temperature change in *Ostrea edulis*, *Oecologia* **30**, 1977, 97–110.

Newell R.C. and Pye V.I., The influence of thermal acclimation on the relation between oxygen consumption and temperature in *Littorina littorea* (L.) and *Mytilus edulis* L, *Comp. Biochem. ~~Physiol~~Physiol.* **34**, 1970, 385–397.

Peck L.S., Feeding, metabolism and metabolic scope in Antarctic marine ectotherms, In: Pörtner H.O. and Playle R., (Eds.), *Cold Ocean Physiology,* 1998, Cambridge University Press; Cambridge, 365–390.

Peck L.S., Ansell A.D., Webb K.E., Hepburn L. and Burrows M., Movements and burrowing activity in the Antarctic bivalve molluscs *Laternula elliptica* and *Yoldia eightsi*, *Polar Biol.* **27**, 2004, 357–367, (-367).

Pernet F., Tremblay R., Comeau L. and Guderley H., Temperature adaptation in two bivalve species from different thermal habitats: ~~Energetics~~energetics and remodelling of membrane lipids, *J. Exp. ~~Biol~~Biol.* **210**, 2007, 2999–3014.

Pernet F., Tremblay R., Redjah I., Sévigny J.-M. and Gionet C., Physiological and biochemical traits correlate with differences in growth rate and temperature adaptation among groups of the eastern oyster *Crassostrea virginica*, *J. Exp. Biol.* **211**, 2008, 969–977.

Pörtner H.O., Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems, *J. Exp. Biol.* **213**, 2010, 881–893.

Pörtner H.O., Bennett A.F., Bozinovic F., Clarke A., Lardies M.A., Lenski R.E., Lucassen M., Pelster B., Schiemer F. and Stillman J.H., Trade-offs in thermal adaptation: the need of a molecular to ecological integration, *Physiol. Biochem. Zool.* **79**, 2006, 295–313.

Precht H., Concepts of temperature adaptation of unchanging reaction systems of cold-blooded animals, In: Prosser C.L., (Ed), *Physiological Adaptation,* 1958, American Physiological Society; ~~Washington D.C~~Washington, DC, 51–78.

Pynnönen K.S. and Huebner J., Effects of episodic low pH exposure on the valve movements of the freshwater bivalve *Anodonta cygnea* L, *Water Res.* **29**, 1995, 2579–2582.

Resgalla C., ~~Jr~~Jr., Brasil E.d.S. and Salomao L.C., The effect of temperature and salinity on the physiological rates of the mussel *Perna perna* (Linnaeus 1758), *Brazil. Arch. Biol. Technol.* **50**, 2007, 543–556.

Riascos J.M., Avalos C.M., Pacheco A.S. and Heilmayer O., Testing stress responses of the bivalve *Protothaca thaca* to El Nino-La Nina thermal conditions, *Mar. Biol. Res.* **8**, 2012, 654–661.

Rodland D.L., Schone B.R., Baier S., Zhang Z., Dreyer W. and Page N.A., Changes in gape frequency, siphon activity and thermal response in the freshwater bivalves *Anodonta cygnea* and *Margaritifera falcata*, *J. Molluscan ~~Stud~~Stud.* **75**, 2009, 51–57.

Rome L.C., Influence of temperature on muscle recruitment and muscle function ~~in vivo~~in vivo, *Am. J. ~~Physiol, Reg.,~~Physiol. Reg. Int. Comp. ~~Physiol~~Physiol.* **259**, 1990, R210–R222.

Rome L.C., Influence of temperature on muscle properties in relation to swimming performance, In: Mommsen T.P. and Hochachka P.W., (Eds.), *Biochemistry and Molecular Biology of Fishes, Environmental and Ecological Biochemistry,* 1995, Elsevier; Amsterdam, 73–99.

Scott M., Berrigan D. and Hoffmann A.A., Costs and benefits of acclimation to elevated temperature in *Trichogramma carverae*, *Entomol. Exp. Appl.* **85**, 1997, 211–219.

Sidell B.D., Cellular acclimatisation to environmental change by quantitative alterations in enzymes and organelles, In: Cossins A.R. and Sheterline P., (Eds.), *Cellular Acclimatisation to Environmental Change,* 1983, Cambridge University Press; London, 103–120.

Sukhotin A.A., Respiration and energetics in mussels (*Mytilus edulis* L.) cultured in the White Sea, ~~*Aquacul*~~*Aquaculture* **101**, 1992, 41–57.

Tankersley R.A. and Dimock R.V., Jr, The effect of larval brooding on the respiratory physiology of the freshwater unionid mussel *Pyganodon cataracta*, *Am. Midl.* ~~*Nat.*~~*Nat.* **130**, 1993, 146–163.

Tattersall G.J., Sinclair B.J., Withers P.C., Fields P.A., Seebacher F., Cooper C.E. and Maloney S.K., Coping with ~~thermal challenges:~~Thermal Challenges: Physiological ~~adaptations~~Adaptations to ~~environmental temperatures.~~Environmental Temperatures. Comprehensive Physiology, 2012, John Wiley & Sons, Inc.

Tudorancea C. and Florescu M., Considerations concerning the production and energetics of *Unio tumidus* Philipsson population from the *Crapina marsh*, ~~*Travaux du Muséum National d'Histoire Naturelle*~~ *Trav. Mus. Nat.Hist. Nat. "Grigore Antipa"* **8**, 1968, 395–405.

Urrutia M.B., Ibarrola I., Iglesias J.I.P. and Navarro E., Energetics of growth and reproduction in a high-tidal population of the clam *Ruditapes decussatus* from Urdaibai Estuary (Basque Country, N. Spain), *J. Sea Res.* **42**, 1999, 35–48.

Watters G.T., A ~~brief look~~Brief Look at ~~freshwater mussel~~Freshwater Mussel (Unionacea) ~~biology,~~Biology. Freshwater Bivalve Ecotoxicology, 2007, CRC Press; Boca ~~Raton~~Raton, FL, 51–64.

Widdows J., The effects of temperature on the metabolism and activity of *Mytilus edulis*, *Neth. J. Sea Res.* **7**, 1973, 387–398.

Widdows J., Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*, *J. Mar. Biol. Assoc.* ~~*U.K*~~*U.K.* **58**, 1978, 109–124.

Wilson, R., 2007. Testing the benefits of acclimation to reproductive performance in male ~~mosquitofish, in:~~mosquitofish. In: Mommsen, T.P., Walsh, P.J. (Eds.), SEB Main Meeting, Glasgow, p. S205.

Wilson R.S. and Franklin C.E., Testing the beneficial acclimation hypothesis, *Trends Ecol. Evol.* **17**, 2002, 66–70.

Woods H.A. and Harrison J.F., Interpreting rejections of the beneficial acclimation hypothesis: ~~When~~when is physiological plasticity adaptive?, ~~*Evo*~~*Evolution* **56**, 2002, 1863–1866.

Appendix A. Supporting information

Multimedia Component 1

Fig. S1 **(i don't know if the end figure will be that small, but when I click on the link, the figure presented is no bigger than a postage stamp and impossible to read.)**

Multimedia Component 2

Supplementary material

Highlights

- The freshwater mussel *Unio tumidus* experiences significant seasonal temperature variation.
- Burrowing rate and valve closure behaviour vary directly with temperature.
- Oxygen consumption also varies directly with temperature.
- There is little evidence of thermal compensation in *Unio tumidus*.
- The costs of compensation in a low-energy species like *Unio tumidus* must outweigh the benefits.

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Queries and Answers

Query: Please confirm that given names and surnames have been identified correctly and are presented in the desired order.

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